Identification of False-Positive Syphilis Antibody Results Using a Semiquantitative Algorithm[▽]

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Screening patients for syphilis serology using a "treponemal assay-first" approach presents unique challenges, particularly when applied to low-prevalence populations. The use of a screening algorithm that incorporates semiquantitative values from treponemal antibody test results can help to identify potential false-positive results while requiring a minimum of repeat testing.

Syphilis is a sexually transmitted disease caused by infection with the spirochete *Treponema pallidum*. Because of an inability to routinely culture the infectious agent, diagnosis of syphilis infection is primarily done by a combination of clinical presentation and serology. This serologic testing can be broadly divided into two types of assays: treponemal tests that test for antibodies directed against *T. pallidum* and nontreponemal tests that measure anticardiolipin antibodies produced during active infection.

In recent years, many laboratories have shifted to screening patients with a treponemal assay and then reflexing positive samples to nontreponemal testing, a practice which reverses the historical approach. There is ongoing debate in the literature about the relative merits of the algorithms. While a portion of the discussion focuses on the clinical relevance of identifying treponemal antibodies in asymptomatic patients and the cost-effectiveness of different screening algorithms (4, 8, 9), an additional concern is the analytical performance of treponemal tests available on the market (10). Although initial studies suggested that the rate of analytical false-positive results was relatively low when using treponemal antibody tests for screening (3), a more recent survey identified a higher frequency of unconfirmed positive results (2). However, these results are somewhat difficult to interpret, as several different combinations of screening and confirmatory tests were used. While many studies have compared the performance characteristics of various marketed treponemal assays (1, 5, 7) one limitation is that treponemal serology results are usually considered to represent a binary variable (i.e., "reactive" versus "nonreactive") rather than a continuous one. To address this, we examined whether semiquantitative results provide additional information relevant to determining a patient's serologic status.

Samples were analyzed for the presence of treponemal antibodies by the use of two immunoassays: the Bioplex 2200 syphilis IgG assay (SYPHG) (Bio-Rad Laboratories, Hercules, CA) and the Trep-Sure assay (Phoenix BioTech Corp., Oakville, Ontario, Canada). The Bioplex SYPHG assay is a bead-based multiplex immunoassay that uses recombinant treponemal antigens (Tp15, Tp17, and Tp47) as the capture reagent, followed by detection with a murine anti-human IgG-phycoerythrin (PE) conjugate (6). Results are expressed as an "antibody index" (AI), which is an arbitrary unit related to the ratio of sample signal to calibrator-defined cutoffs. Trep-Sure is a microplate-based enzyme immunoassay (EIA) that also uses recombinant treponemal antigens (in a proprietary mixture) as the capture reagent but utilizes peroxidase-conjugated treponemal antigens for detection (11). Nontreponemal antibody measurement was performed by rapid plasma reagin (RPR) testing (Becton Dickinson, Franklin Lakes, NJ).

Study specimens were selected from samples sent to our laboratory for routine syphilis testing and analyzed without knowledge of clinical histories. Aliquots from samples that were reactive in the initial Bioplex SYPHG screening were further tested by both the Trep-Sure EIA and RPR. Our laboratory serves a low-prevalence population, with an initial screen-positive rate of approximately 3% based on historical data (data not shown).

A total of 142 samples that were identified as reactive in the initial Bioplex screening assay underwent reflex testing as described above. The presence of treponemal antibodies was confirmed by the Trep-Sure EIA in 77% (110/142) of the samples, a rate similar to that reported in earlier multicenter studies (3). However, the likelihood of confirmation was highly dependent on both the patient's RPR status and the SYPHG value determined in the Bioplex screening assay (Fig. 1). Treponemal antibody status was confirmed by EIA for all RPR-positive samples ($n = 27$), regardless of the initial SYPHG value. In contrast, discordant results were determined for 28% (32/115) of the RPR-negative patients, with an increasing frequency in samples with low SYPHG values in the initial screening. Receiver operating characteristic (ROC) analysis was performed to identify a cutoff value that would provide a high level of specificity for identifying "true-positive" (EIA-confirmed) samples (Fig. 2). A cutoff AI value of 6.0, providing 100% specificity (confidence interval [CI], 89.3 to 100.0%), was selected. All samples with screening SYPHG values above this level (78/78) were confirmed by the EIA compared to only 50% (32/64) of the samples with screening SYPHG levels < 6.0 AI ($P < 0.0001$ [Fisher's exact test]).

To ensure that this disparity was not due to differences in

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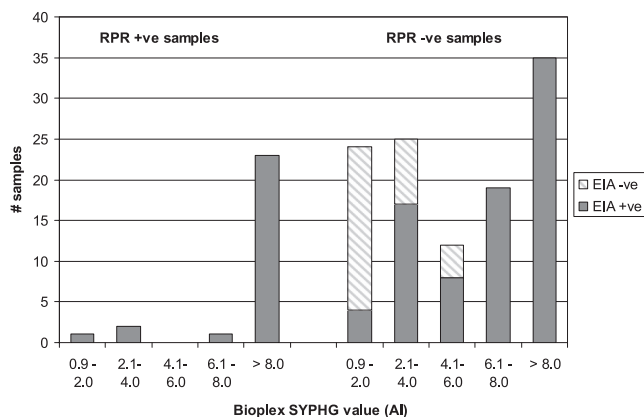
FIG. 1. Comparison of treponemal antibody results. A total of 142 samples were tested for treponemal antibodies by the use of both the Bioplex and Trep-Sure (EIA) assays as well as for RPR reactivity. Results are categorized by the SYPHG value detected in the initial Bioplex screening assay.
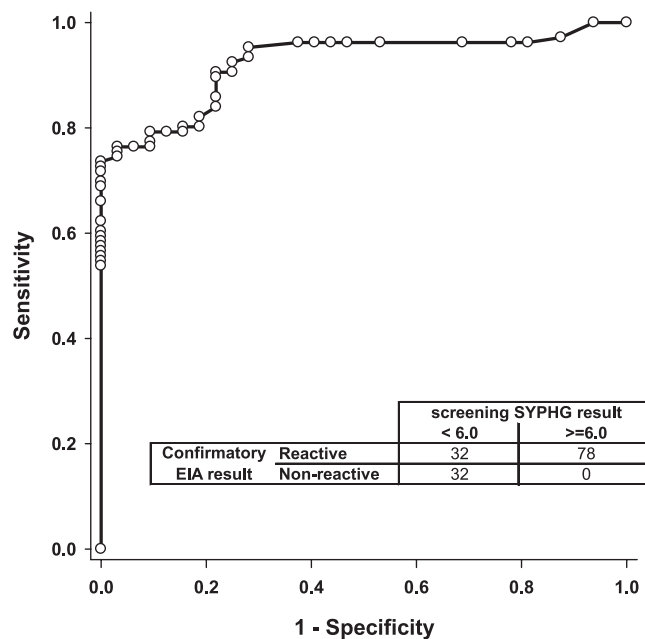

FIG. 2. ROC analysis of SYPHG values for predicting true-positive (EIA-confirmed) samples. Sensitivity was defined as the identification of samples with reactive results in the confirmatory EIA. The value for the area under the curve (AUC) is 0.919 (95% CI, 0.87 to 0.96), with 100% specificity achieved at all AI values ≥ 5.8. A 2-by-2 contingency table constructed using 6.0 AI as the cutoff is also shown. The frequencies of EIA-confirmed results for the two groups were significantly different ($P < 0.0001$ [Fisher's exact test]).

analytical sensitivity between the Bioplex and Trep-Sure assays, five strongly positive samples (SYPHG results of >8.0 AI) were diluted with normal serum to achieve values between 1.0 to 2.0 AI in the Bioplex assay. All five of these diluted samples gave positive results in the Trep-Sure assay, demonstrating that this confirmatory test was capable of detecting treponemal antibodies at these lower concentrations. This was consistent with the results seen in RPR-positive samples, where several weakly positive specimens were nonetheless confirmed by EIA.

Determining the relevance of a positive treponemal antibody result for a patient with a negative RPR can be challenging, particularly in the absence of a clear clinical history. For this reason, it is critical to identify and minimize analytical false-positive results. In this report, we demonstrate that the SYPHG value can help to inform this decision and can be used when developing a strategy for reflex testing. Although the screening assay used in this study has not been cleared by the FDA as a quantitative assay, these data suggest that the semiquantitiative AI result can still provide useful information related to the need for confirmatory testing. Based on this data, we offer the following algorithm for syphilis screening at our institution. All samples that are reactive in the initial SYPHG screening test are further tested by RPR. RPR-positive samples require no further testing, regardless of the initial AI in the SYPHG assay. In contrast, RPR-negative tests with initial SYPHG results of <6.0 are automatically subjected to confirmatory EIA. This algorithm limits reflex EIA testing to roughly 1% of samples sent for syphilis testing but focuses on the samples most likely to have discordant results.

One limitation is that, because of a lack of standardization of treponemal assays from different manufacturers, the specific SYPHG AI values identified in this study apply only to the cases where initial screening is performed using the Bioplex assay. However, the approach demonstrated here can be readily evaluated for other assays in order to determine the ideal cutoff value to trigger reflex testing. An important point is that, when confirmatory testing is implemented, it must be done using an assay that has an analytical sensitivity for detecting low concentrations of treponemal antibodies equal to or better than that of the screening assay. A systematic analysis of the relative analytical sensitivities of current treponemal assays would be a valuable tool to help laboratories establish appropriate testing algorithms.

Determining the relative merits of using treponemal versus nontreponemal assays to evaluate patients for syphilis infection requires further study. However, regardless of the method, laboratories should develop approaches to identify analytical false-positive results wherever possible. Understanding the analytical performance characteristics of treponemal assays, together with establishing assay-specific cutoffs to trigger confirmatory testing, is an approach that can be used to help in this regard, particularly when screening low-prevalence populations.

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